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Supporting Information

Molecular mechanism of green tea polyphenol Epicatechin gallate attenuating *Staphylococcus aureus* pathogenicity by targeting Ser/Thr phosphatase Stp1

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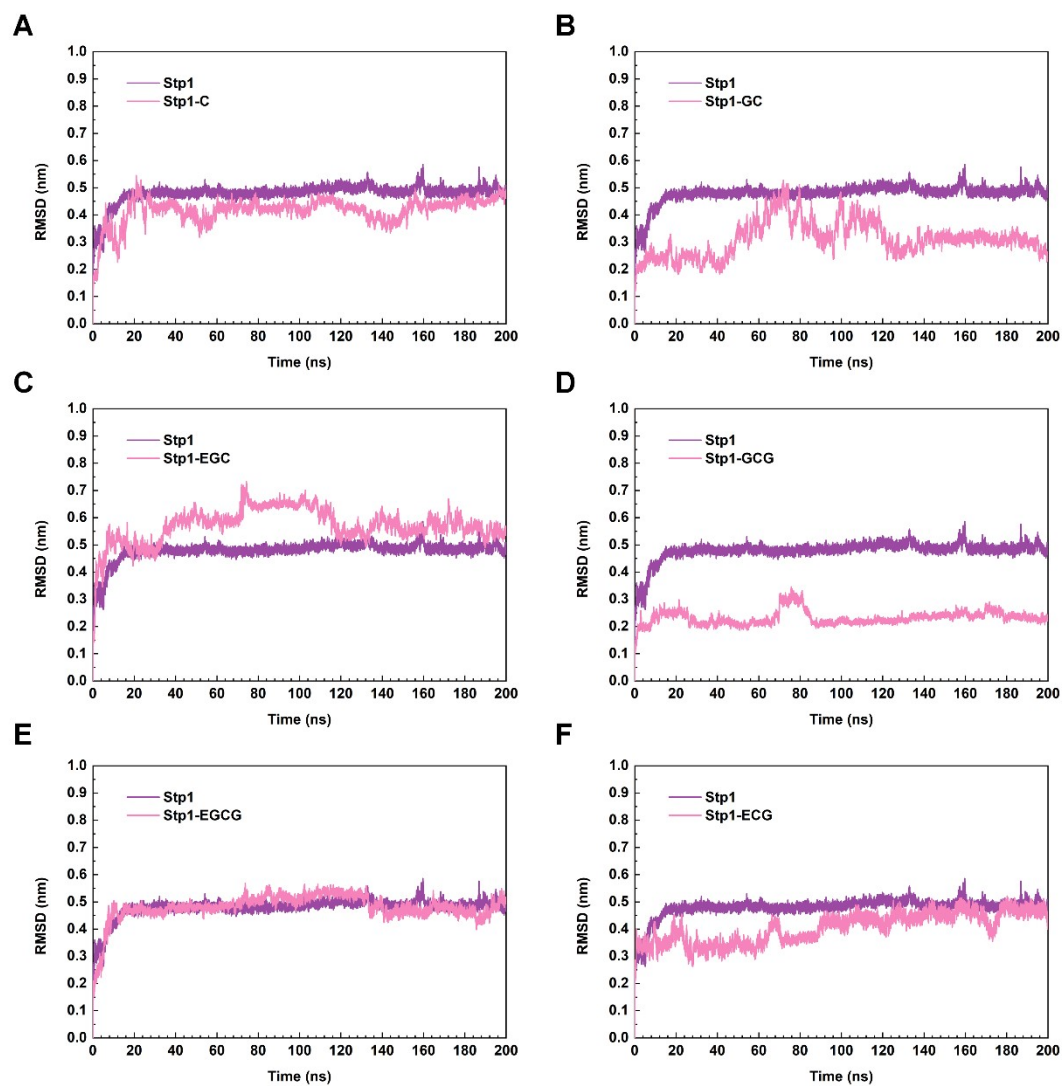
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18 Figures and tables

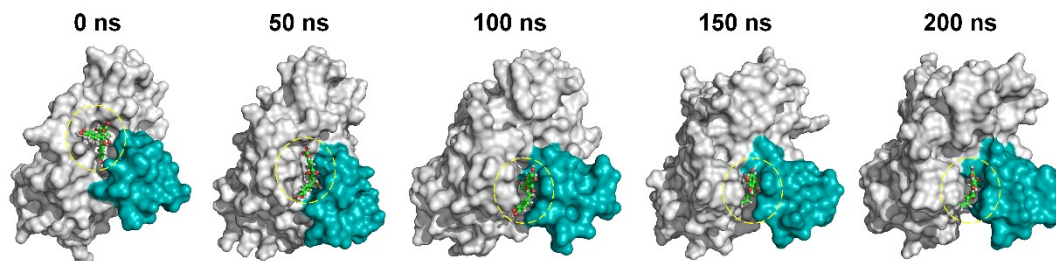


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20 **Fig. S1** The RMSD displayed by the backbone atoms of Stp1 during MD simulations

21 of free protein and Stp1-ligand complexes.

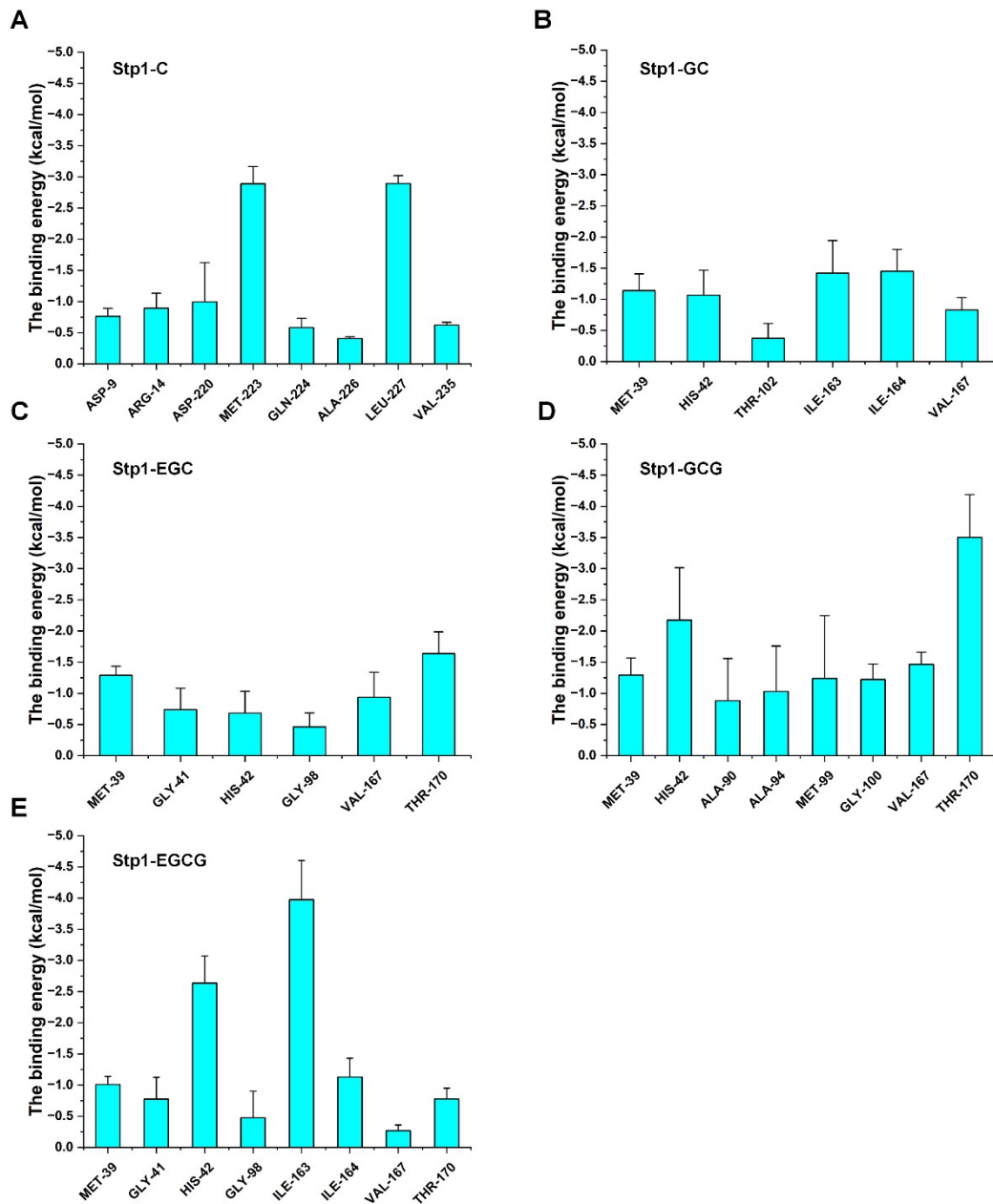
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24 **Fig. S2** The migration of ECG during MD simulations.

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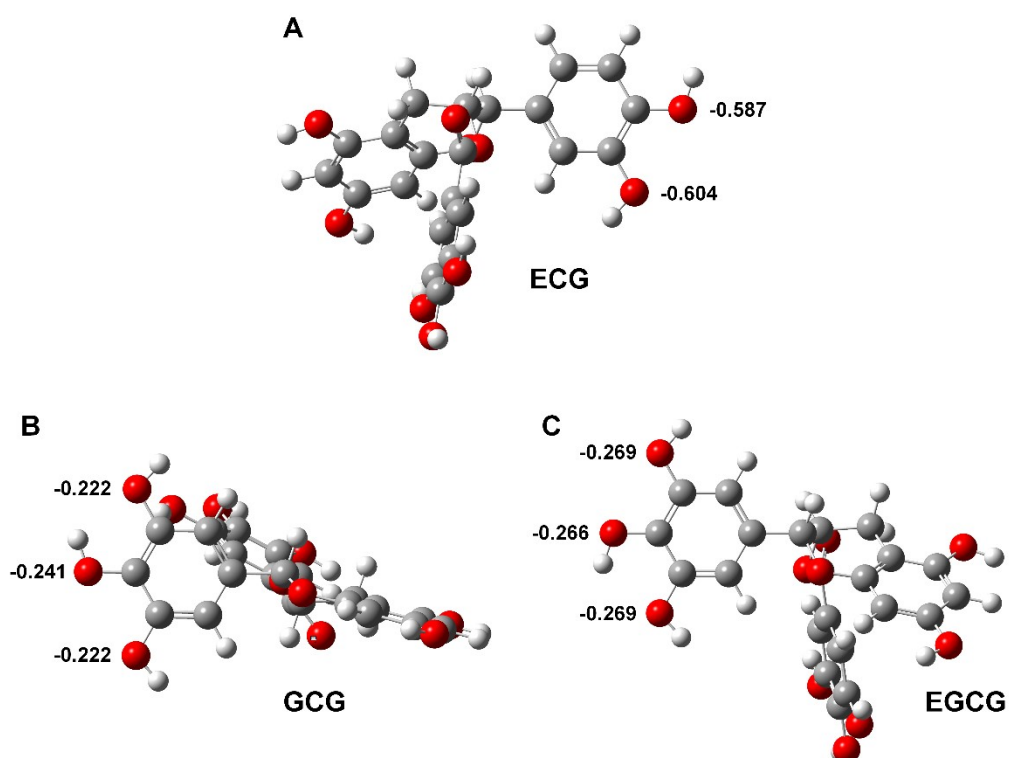


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27 **Fig. S3** Decomposition of the binding energy on residues in the binding sites of Stp1-

28 ligand complex models.

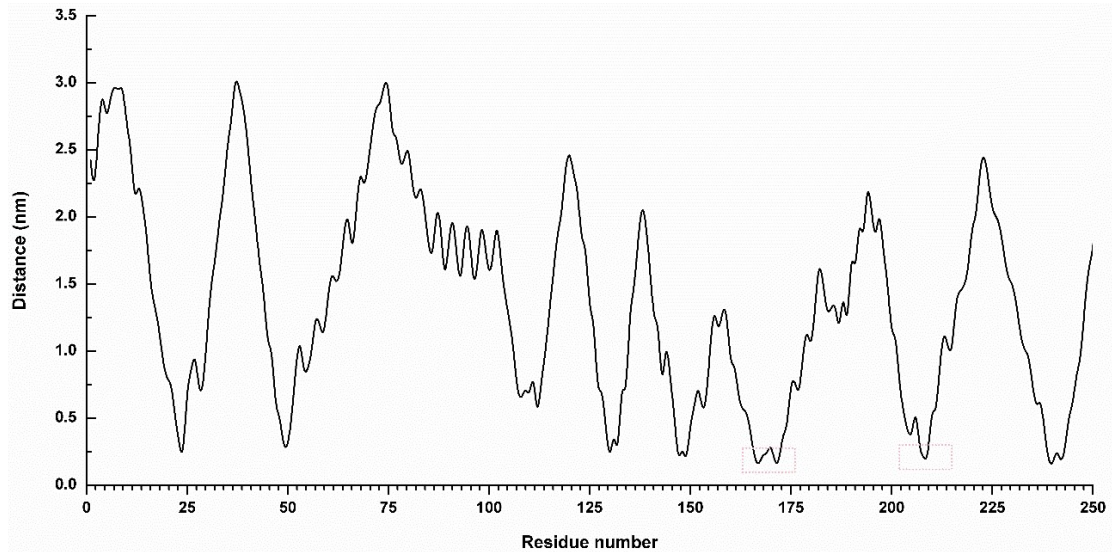
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31 **Fig. S4** The charge distribution of oxygen atoms on GCG, EGCG, and ECG.

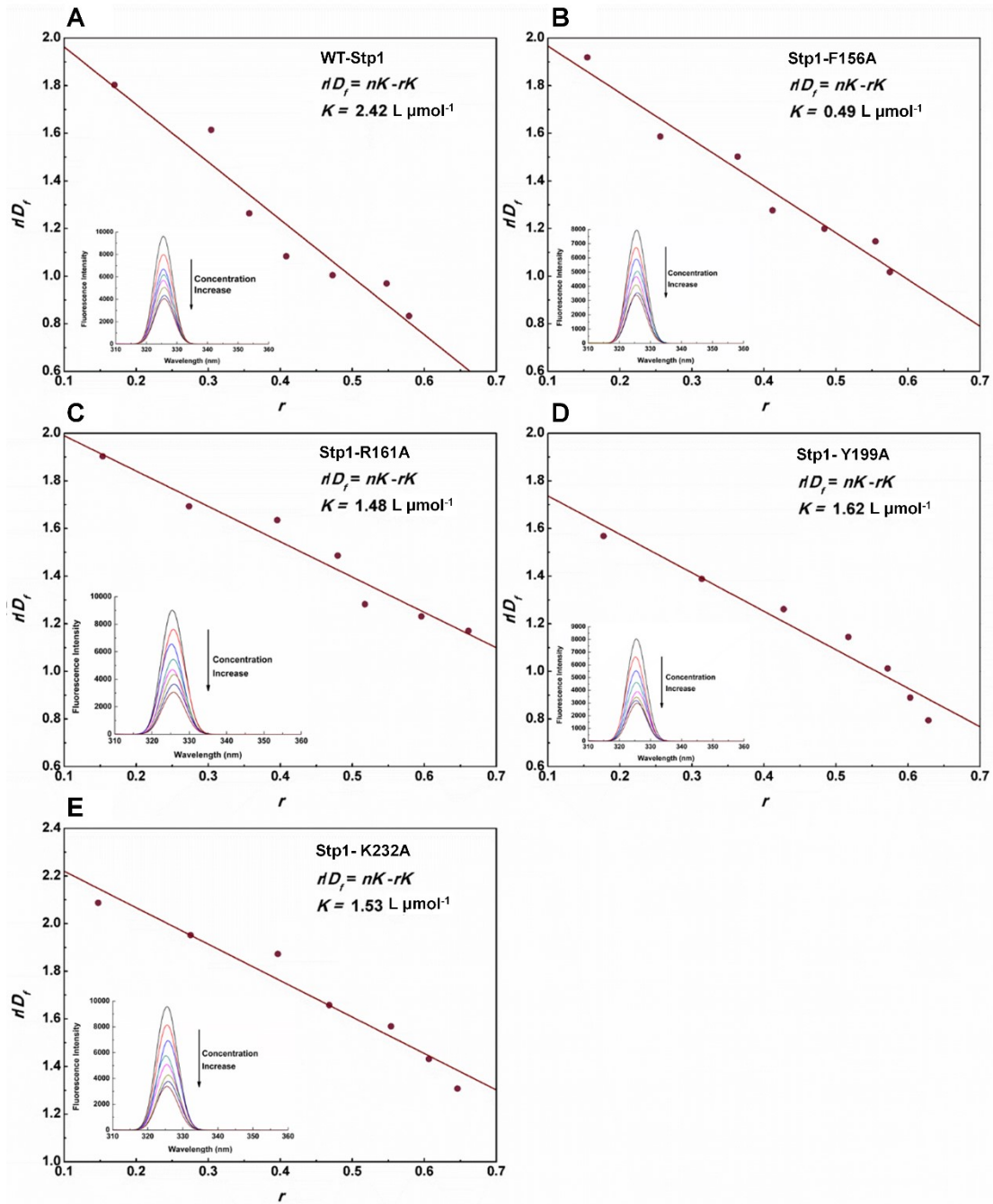
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34 **Fig. S5** The distance between the whole residues of Stp1 and ECG.

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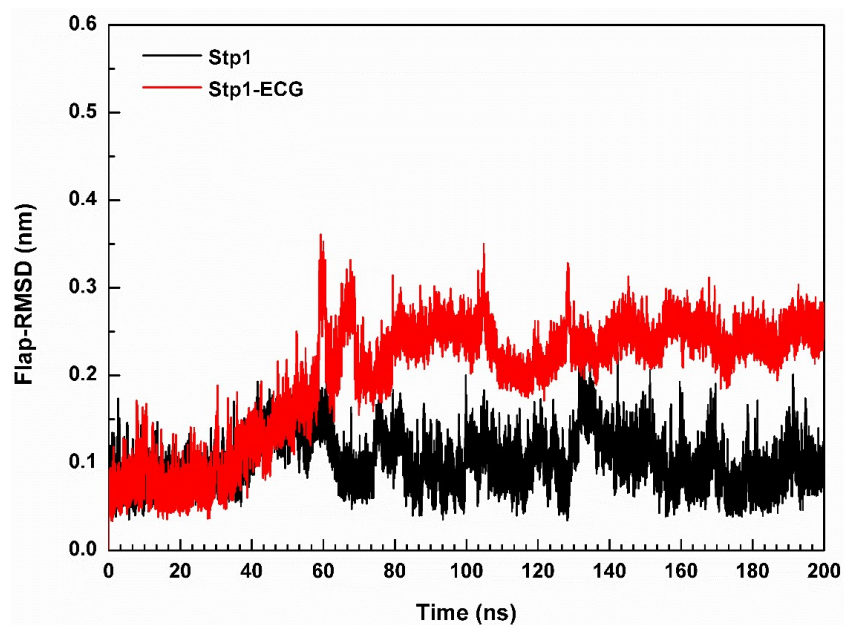
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37 **Fig. S6** The Scatchard plots of r/D_f vs. r for ECG binding to WT-Stp1 (A), Stp1-F156A

38 (B), Stp1-R161A (C), Stp1-Y199A (D), and Stp1-K232A (E). inset: Effects of ECG on

39 fluorescence spectra of Stp1 with the concentration increase.

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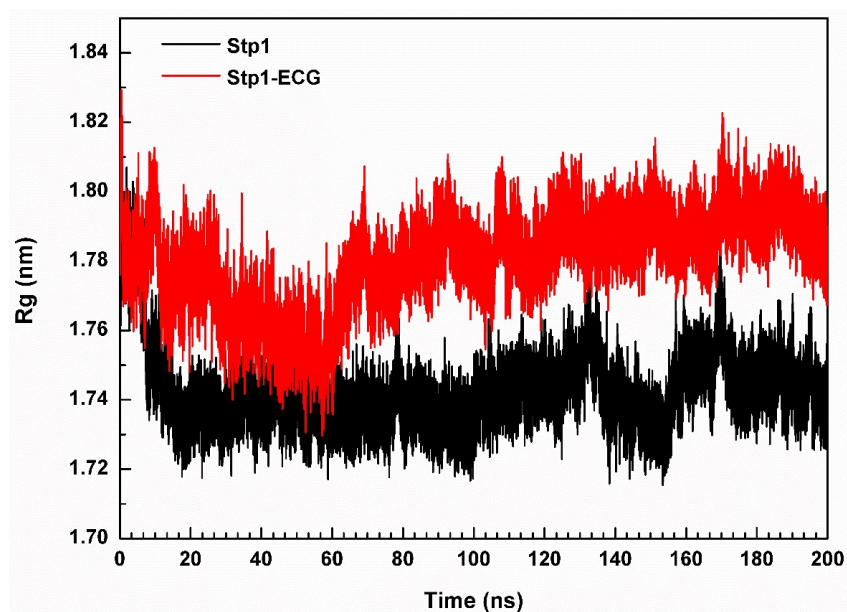


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42 **Fig. S7** RMSD values of the backbone atoms of flap subdomains in free Stp1 and Stp1-

43 ECG complex.

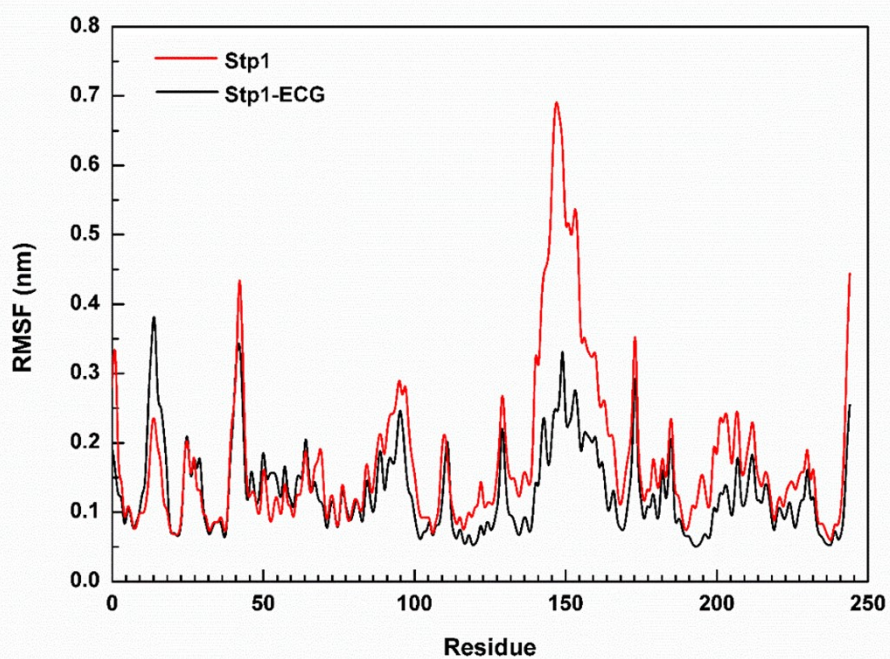
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46 **Fig. S8** Radius of gyration (Rg) values of backbone atoms in free Stp1 and Stp1-ECG
47 complex.

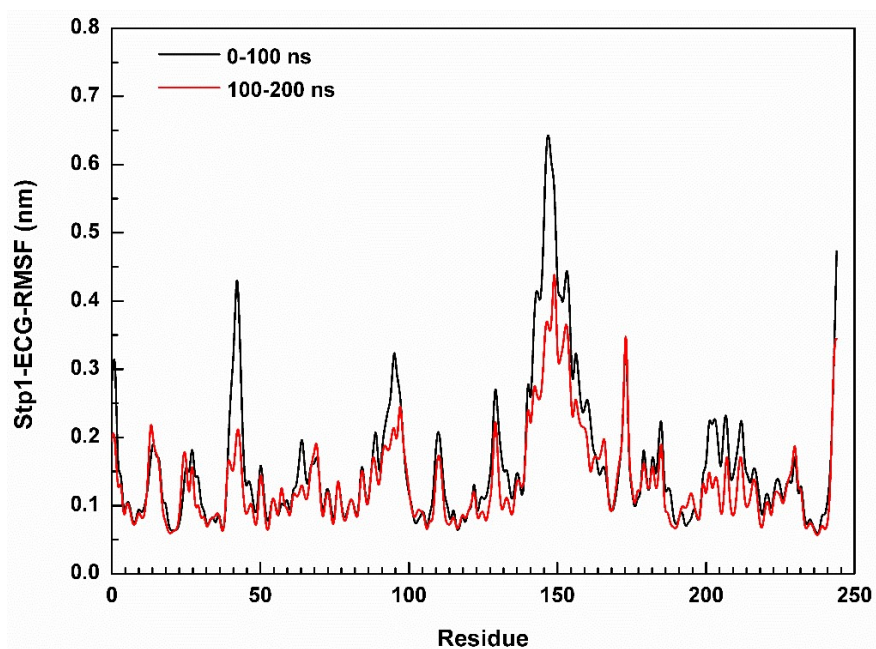
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50 **Fig. S9** RMSF of all the residues in free Stp1 and Stp1-ECG complex.

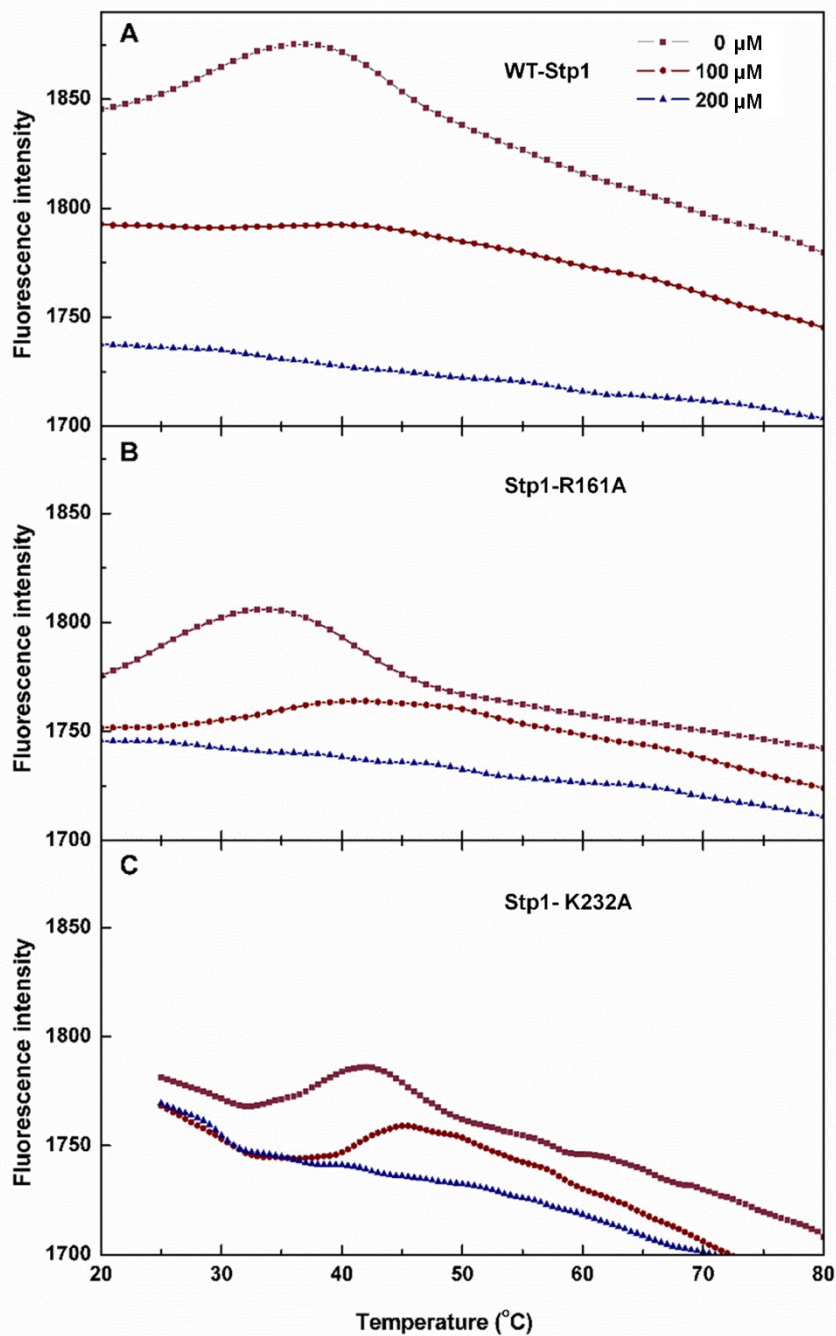
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53 **Fig. S10** RMSF values of the whole residues in Stp1-ECG complex during 0-100 ns
54 and 100-200 ns.

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57 **Fig. S11** The T_m of Stp1 and mutants via thermal shift assay. The effects of various
 58 amounts of ECG on the fluorescence curves of the WT-Stp1 (A), R161A (B), and
 59 K232A (C) variants.

60

61 **Table S1** Primers used for mutagenesis in this study.

Primers	Sequence (5'-3')
Stp1F156A-F	CCGGAAGAAGCAGCGACACATCCACAAC
Stp1F156A-R	GTTGTGGATGTGTCGCTGCTTCTTCCGG
Stp1R161A-F	CACATCCACAAGCGAATATTATTAC
Stp1R161A-R	GTAATAATATTCGCTTGTGGATGTG
Stp1Y199A-F	GATGGATTAAGTATGCGGTAAAGACAATGA
Stp1Y199A-R	TCATTGTCTTTAACCGCATCAGTTAATCCATC
Stp1K232A-F	GATAACCATTTCGGCGGATAACGTTAC
Stp1K232A-R	GTAACGTTATCCGCCGAATGGTTATC

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63 **Table S2** Primers used for qRT-PCR in this study.

Primers	Sequence (5'-3')
<i>16S -F</i>	ACCAGTGGCGAAGGCGACTTT
<i>16S-R</i>	CAGGCGGAGTGCTTAATGCGTTA
<i>hla-F</i>	GCGGCCTTATTGGTGCAAAT
<i>hla-R</i>	TGCCATATACCGGGTTCCAAG
<i>hld-F</i>	GGAGTGATTTCAATGGCACAAGAT
<i>hld-R</i>	GTGAATTTGTTCACTGTGTCGATAATCC
<i>PSM β2-F</i>	ATGACTGGACTAGCAGAAGCAATCG
<i>PSM β2-R</i>	ACCTAGTAAACCCACACCGTTAGCA

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65 **Table S3** The binding free energy (kcal mol⁻¹) of ECG with WT-Stp1 and mutants

66 based on MM-PBSA method.

Energy	WT	F156A	K232A	R161A	Y199A
Component					
ΔE_{vdw}	-36.12 ± 3.43	-42.83 ± 4.19	-42.57 ± 3.97	-40.13 ± 4.29	-39.96 ± 3.68
ΔE_{ele}	-58.62 ± 8.84	-74.45 ± 7.53	-61.98 ± 8.28	-59.18 ± 8.59	-61.37 ± 8.44
$\Delta G_{ele,sol}$	67.69 ± 8.51	94.22 ± 6.79	78.41 ± 6.78	76.79 ± 7.19	78.48 ± 6.65
$\Delta G_{nonpolar,sol}$	-5.65 ± 0.14	-6.04 ± 0.16	-5.56 ± 0.17	-5.83 ± 0.20	-5.99 ± 0.18
ΔG_{gas}	-94.74 ± 8.97	-117.28 ± 7.53	-104.54 ± 7.65	-99.31 ± 8.77	-101.32 ± 7.03
ΔG_{sol}	62.04 ± 8.46	88.19 ± 6.70	72.85 ± 6.79	70.97 ± 7.13	72.49 ± 6.65
ΔG_{bind}	-32.70 ± 4.06	-29.09 ± 2.88	-31.70 ± 3.15	-28.34 ± 5.44	-28.84 ± 3.75

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